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PATENT
09/995,419
Docket 096/004

REMARKS

This paper is responsive to the Office Action dated December 16, 2003 (Paper No. 12), which is the first action on the merits of the application.

Claims 1-22 were previously pending in the application; claims 1-15 were under examination. Upon entry of this Amendment, claims 16-22 are canceled, and claims 23-47 are added. The added claims fall within the group under examination. Accordingly, claims 1-15 and 23-47 are now pending in the application and under examination.

Further consideration and allowance of the application is respectfully requested.

Interview:

Applicant thanks Examiner Joseph Weitach for the courtesy of an interview with Michael Schiff at the Patent Office on March 11, 2004. Recommendations made by the examiner have been incorporated into the claim amendments and remarks presented herein. The application is now believed to be in condition for allowance.

Claim amendments:

Claims in the application have the following features:

- Independent claim 14 and claims depending therefrom cover methods for producing a cell population by genetically altering undifferentiated stem cells in a population, and then causing at least some of the cells to differentiate. The genetic alteration enables undifferentiated stem cells to be depleted, if desired, in an optional subsequent step (claim 15).
- Independent claim 1 and claims depending therefrom cover methods for depleting undifferentiated stem cells from a mixed cell population. Undifferentiated cells in the population are genetically altered, and then the undifferentiated cells are depleted.
- Independent claim 31 and claims depending therefrom cover methods for preparing a cell population in a manner that parallels claim 14, but limited to hES cells and their equivalents.
- Independent claim 43 and claims depending therefrom cover methods for depleting undifferentiated cells in a manner that parallels claim 1, but limited to hES cells and their equivalents.

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The claim amendments do not add new matter to the disclosure. The use of adenovirus for making a transient genetic alteration (claim 23), and retrovirus or DNA plasmids for making an inheritable alteration (claim 24), is illustrated in Example 5 (page 31 ff.), and Example 7 (page 33 ff.). The use of an endogenous or heterologous promoter for driving expression (claims 32-33) is supported on page 5, lines 21-23. Differentiation of hES cells into neural cells or hepatocytes is described in the specification in page 17, line 29 to page 18, line 3. Formulating the differentiated cells for administration to a mammalian host (the final procedure in claims 31 and 43) is described in the section on page 25.

The other new claims are supported by claims 1-15 as previously presented. It is understood that the claims permit but do not require that all of the cells in the respective cell population be genetically altered in the manner specified. What the claims indicate is that some undifferentiated stem cells in the population are genetically altered, such that expression of the new cell surface antigen can be used effectively to remove some of the stem cells at a subsequent time, if performed or desired.

The subject matter of cancelled claims 17-22 will be pursued in a divisional application. Applicant reserves the right to introduce claims in this or any related application for any subject matter previously claimed or described in the specification.

Claim objection

Claim 10 is objected to as not further limiting claim 14 from which it depends.

In fact, claim 14 requires only that the cells *contain* the structure P-X after being genetically altered. As described in the specification on page 5, lines 21 ff., this can be accomplished not only by introducing P-X into the cell as a complete construct, but also by transfecting the cells so as to place a heterologous encoding region (X) next to a promoter (P) that is already present in the genome. The skilled reader will appreciate that there are other ways of constructing P-X in the cell, such as placing a heterologous promoter (P) next to an endogenous encoding region (X), or introducing a heterologous P and X into the cell as separate constructs so that they ultimately become operatively linked.

Claim 10 further limits claim 14 by explicitly indicating that P-X is introduced into the cell as a single construct.

Rejection under 35 USC § 112 ¶ 1:

Claim 15 and 5-8 stand rejected under the enablement requirements of § 112 ¶ 1. The Office Action indicates that the specification generally enables the making and use of cells genetically altered

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with a heterologous construct that expresses a cell surface antigen in undifferentiated cells. However, it indicates that the use of glycosyltransferases and complement to lyse undifferentiated cells requires undue experimentation.

Applicant respectfully disagrees. All of the components needed to process cell populations according to these claims can be performed using commercially available reagents. Design and optimization of the experimental protocol falls well within the routine experimentation allowed by the *Wands* standard.

1. Selection of glycosyltransferase.

Many glycosyltransferases made by vertebrates are exquisitely specific: they transfer only a single type of sugar substrate in only one position and orientation to an exact acceptor structure recognized by the enzyme several monosaccharides deep. Glycosyltransferases are in this respect at least as specific as antibody molecules. The specificity is necessary, because they build elaborate and well-defined structures that in turn provide recognition sites for adhesion molecules implicated in embryogenesis, tissue growth and repair, lymphocyte migration and other intercellular recognition events. There are about 50 c-type lectins and 10 galectins known to be involved in binding activity mediated by cell surface carbohydrates (Gorelik et al., *Cancer Metastasis Rev.* 2001;20:245-77).

Exemplary is $\alpha(1,3)$ galactosyltransferase (claim 7). It uses UDP-galactose as a source of galactose, which it transfers specifically to an acceptor oligosaccharide, usually $\text{Gal}\beta(1,4)\text{GlcNAc}$ (N-acetyl lactosamine). All mammals express this enzyme, except for catharines (upper primates, including humans). In humans, $\alpha(1,2)$ fucosyltransferase builds the N-acetyl lactosamine into $\text{Fuca}(1,2)\text{Gal}\beta(1,4)\text{GlcNAc}$, which is blood group H substance. This in turn serves as an acceptor substance for blood group A GlcNAc-transferase, or blood group B Gal-transferase (claim 8), forming A-substance or B-substance, respectively, depending on the blood type of the individual. A number of genes for other glycosyltransferases involved in forming blood group antigens or other cell surface carbohydrates are available (see Appendix A).

Blood group carbohydrates appear on most cell types in the body, including stem cells. Accordingly, sequences encoding $\alpha(1,3)$ galactosyltransferase, blood group A transferase, and blood group B transferase would all be useable, or any other glycosyltransferase enzyme forming a defined carbohydrate antigen not normally present in the cell population being processed.

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2. Obtaining antibody or lectin against the determinant formed by the glycosyltransferase

Because each glycosyltransferase makes an exact carbohydrate structure with its own antigenic features, it is relatively easy to make antibody that will specifically recognize it. As indicated in the specification, there are often naturally occurring antibodies present in the circulation of mammals against carbohydrate structures that are not self-antigens (page 21, line 17 ff.). This is thought to occur because of cross-reactive carbohydrate determinants present in the diet. Since the cells of claims 5-8 are engineered to express a new carbohydrate determinant, human serum may be a ready source of antibody to perform the depletion step. If not, then specific monoclonal or polyclonal antibody can be raised according to standard techniques (specification page 21, lines 3-16).

Alternatively, there is a wide range of lectins commercially available that are highly specific for particular carbohydrate determinants, such as blood group substances (See Appendix B).

3. Depleting cells expressing the carbohydrate determinant

As indicated in the specification, depletion of undifferentiated cells according to claims 15 or 6-8 can be effected using a variety of separation techniques mediated by specific antibody or lectin: for example, immunoadsorption or fluorescence activated cell sorting (page 21, line 30 ff.). In fact, a number of lectins specific for blood group determinants come already labeled with a fluorochrome such as FITC or TRITC, or linked to a solid support like agarose (Appendix B).

Another suitable technique is complement-mediated lysis (claim 5). This is performed by combining the cells with antibody and a source of complement, and simply incubating to effect lysis.

The skilled reader will know that most sources of mammalian complement will work, because of the events that initiate complement lysis. Binding of antigenic determinants by specific IgG antibody via the IgG Fab region activates complement component C1q through the IgG Fc region. This initiates the classic activation cascade, which forms the membrane attack complex and causes lysis. The binding of C1q to IgG is not species specific.

For this reason, guinea pig serum is often used as a source of complement with antibody from other species (Appendix C), even though specific antibody is rarely made in guinea pigs. Fresh human serum is also a suitable source. Where the cells have been modified to express a blood group antigen (claims 7-8), human serum can be used to provide both the antibody and the complement at the same time.

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Applicant respectfully submits that depletion of undifferentiated stem cells expressing glycosyltransferase using specific antibody and complement can be performed with standard reagents using standard techniques. Withdrawal of this rejection is requested.

Rejections under 35 USC § 112 ¶ 2:

Claims 1, 10, 11, 12, and 13 stand rejected for being indefinite or unclear.

Claims 1, 10, and 11 have been amended in a manner that is believed to overcome the concerns raised in the Office Action. Definition of what is meant by an "endogenous" promoter on page 10, lines 36-41 resolves the issue raised with respect to claim 11.

Claims 12 and 13 are rejected for referring to genetic alterations that are transient or permanent. The meaning of these terms is explained on page 22, lines 28-25; and page 10, line 16. Use of adenovirus for making a transient genetic alteration, and retrovirus or DNA plasmids for making an inheritable alteration, is illustrated in Example 5 (page 31 ff.), and Example 7 (page 33 ff.).

Withdrawal of these rejections is respectfully requested.

Rejections under 35 USC § 102:

Claims 1-4, 9-10, and 14-15 stand rejected as being anticipated by U.S. Patent 6,146,888 (Smith et al.). The Office Action indicates that the reference focuses on isolation of stem cells [not differentiated cells], but the remaining population would be considered depleted of stem cells.

Applicant does not agree. Since the reference is focused on removing differentiated cells from stem cell populations, its objective is opposite of this invention, which is to enable removal of stem cells from differentiated cell populations. The "remaining" differentiated cells would be discarded, and neither Smith et al. nor the skilled reader can truly be said to be placed in *possession* of the invention claimed here in a manner that adequately supports a § 102 rejection.

By way of this amendment, the claims have been amended to provide a further distinguishing feature. Claims 1 and 14 now indicate that after depleting or differentiating the cells, the differentiated cells are then subject to further culturing. In contrast, Smith et al. is focused on things that can be done with the undifferentiated cells, and there is no motivation to recover the differentiated cells for culturing or any other purpose.

New independent claims 31 and 43 are distinguished from Smith et al. for the same reason. Since Smith et al. is focused on things that can be done with the undifferentiated cells, there is no motivation to recover the differentiated cells and administer them to a mammalian host.

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Withdrawal of this rejection is respectfully requested.

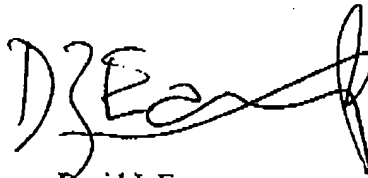
Request for further interview

The application is believed to be in condition for allowance, and a prompt Notice of Allowance is requested.

In the event that the Examiner determines that there are other matters to be addressed, applicant hereby requests an interview by telephone.

Accompanying this Response are papers authorizing the Commissioner to charge the Deposit Account for the fees due. Should the Patent Office determine that a further extension of time or any other relief is required for further consideration of this application, applicant hereby petitions for such relief, and authorizes the Assistant Commissioner to charge the cost of such petitions and other fees due in connection with the filing of these papers to Deposit Account No. 07-1139, referencing the docket number indicated above.

Respectfully submitted,



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April 16, 2004

APPENDIX A:

Listing of glycosyltransferase enzymes according to Enzyme Commission (EC) designation



Get Nucleotide sequences

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Integrated Enzyme Database (IntEnz)

IntEnz**EC 2 Transferases****EC 2.4 Glycosyltransferases****EC 2.4.1 Glycosyltransferases**

Conte

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- [2.4.1.1](#) phosphorylase
- [2.4.1.2](#) dextrin dextranase
- [2.4.1.3](#) *Now included with EC [2.4.1.25](#)*
- [2.4.1.4](#) amylosucrase
- [2.4.1.5](#) dextransucrase
- [2.4.1.6](#) deleted
- [2.4.1.7](#) sucrose phosphorylase
- [2.4.1.8](#) maltose phosphorylase
- [2.4.1.9](#) inulosucrase
- [2.4.1.10](#) levansucrase
- [2.4.1.11](#) glycogen (starch) synthase
- [2.4.1.12](#) cellulose synthase (UDP-forming)
- [2.4.1.13](#) sucrose synthase
- [2.4.1.14](#) sucrose-phosphate synthase
- [2.4.1.15](#) α , α -trehalose-phosphate synthase (UDP-forming)
- [2.4.1.16](#) chitin synthase
- [2.4.1.17](#) glucuronosyltransferase
- [2.4.1.18](#) 1,4- α -glucan branching enzyme
- [2.4.1.19](#) cyclomaltodextrin glucanotransferase
- [2.4.1.20](#) cellobiose phosphorylase
- [2.4.1.21](#) starch synthase
- [2.4.1.22](#) lactose synthase
- [2.4.1.23](#) sphingosine β -galactosyltransferase
- [2.4.1.24](#) 1,4- α -glucan 6- α -glucosyltransferase
- [2.4.1.25](#) 4- α -glucanotransferase
- [2.4.1.26](#) DNA α -glucosyltransferase
- [2.4.1.27](#) DNA β -glucosyltransf rase

- 2.4.1.28 glucosyl-DNA β -glucosyltransferase
- 2.4.1.29 cellulose synthase (GDP-forming)
- 2.4.1.30 1,3- β -oligoglucan phosphorylase
- 2.4.1.31 laminaribiose phosphorylase
- 2.4.1.32 glucomannan 4- β -mannosyltransferase
- 2.4.1.33 alginate synthase
- 2.4.1.34 1,3- β -glucan synthase
- 2.4.1.35 phenol β -glucosyltransferase
- 2.4.1.36 α,α -trehalose-phosphate synthase (GDP-forming)
- 2.4.1.37 fucosylgalactoside 3- α -galactosyltransferase
- 2.4.1.38 β -*N*-acetylglucosaminyglycopeptide β -1,4-galactosyltransferase
- 2.4.1.39 steroid *N*-acetylglucosaminytransferase
- 2.4.1.40 glycoprotein-fucosylgalactoside α -*N*-acetylglactosaminytransferase
- 2.4.1.41 polypeptide *N*-acetylglactosaminytransferase
- 2.4.1.42 *Now included with EC 2.4.1.17*
- 2.4.1.43 polygalacturonate 4- α -galacturonosyltransferase
- 2.4.1.44 lipopolysaccharide 3- α -galactosyltransferase
- 2.4.1.45 2-hydroxyacylsphingosine 1- β -galactosyltransferase
- 2.4.1.46 1,2-diacylglycerol 3- β -galactosyltransferase
- 2.4.1.47 *N*-acylsphingosine galactosyltransferase
- 2.4.1.48 heteroglycan α -mannosyltransferase
- 2.4.1.49 cellodextrin phosphorylase
- 2.4.1.50 procollagen galactosyltransferase
- 2.4.1.51 *Now listed as EC 2.4.1.101, EC 2.4.1.143, EC 2.4.1.144 and EC 2.4.1.145*
- 2.4.1.52 poly(glycerol-phosphate) α -glucosyltransferase
- 2.4.1.53 poly(ribitol-phosphate) β -glucosyltransferase
- 2.4.1.54 undecaprenyl-phosphate mannosyltransferase
- 2.4.1.55 *Now EC 2.7.8.14*
- 2.4.1.56 lipopolysaccharide *N*-acetylglucosaminytransferase
- 2.4.1.57 phosphatidylinositol α -mannosyltransferase
- 2.4.1.58 lipopolysaccharide glucosyltransferase I
- 2.4.1.59 *Now included with EC 2.4.1.17*
- 2.4.1.60 abequosyltransferase
- 2.4.1.61 *Now included with EC 2.4.1.17*
- 2.4.1.62 ganglioside galactosyltransferase
- 2.4.1.63 linamarin synthase
- 2.4.1.64 α,α -trehalose phosphorylase
- 2.4.1.65 3- α -galactosyl-*N*-acetylglu osaminide 4- α -L-fu osyltransferase

- 2.4.1.66 procollagen glucosyltransferase
- 2.4.1.67 galactinol—raffinose galactosyltransferase
- 2.4.1.68 glycoprotein 6- α -L-fucosyltransferase
- 2.4.1.69 galactoside 2- α -L-fucosyltransferase
- 2.4.1.70 poly(ribitol-phosphate) *N*-acetylglucosaminyltransferase
- 2.4.1.71 arylamine glucosyltransferase
- 2.4.1.72 *Now EC 2.4.2.24*
- 2.4.1.73 lipopolysaccharide glucosyltransferase II
- 2.4.1.74 glycosaminoglycan galactosyltransferase
- 2.4.1.75 UDP-galacturonosyltransferase
- 2.4.1.76 *Now included with EC 2.4.1.17*
- 2.4.1.77 *Now included with EC 2.4.1.17*
- 2.4.1.78 phosphopolyprenol glucosyltransferase
- 2.4.1.79 galactosylgalactosylglucosylceramide β -D-acetylgalactosaminyltransferase
- 2.4.1.80 ceramide glucosyltransferase
- 2.4.1.81 flavone 7-*O*- β -glucosyltransferase
- 2.4.1.82 galactinol—sucrose galactosyltransferase
- 2.4.1.83 dolichyl-phosphate β -D-mannosyltransferase
- 2.4.1.84 *Now included with EC 2.4.1.17*
- 2.4.1.85 cyanohydrin β -glucosyltransferase
- 2.4.1.86 glucosaminylgalactosylglucosylceramide β -galactosyltransferase
- 2.4.1.87 *N*-acetyllactosaminide 3- α -galactosyltransferase
- 2.4.1.88 globoside α -*N*-acetylgalactosaminyltransferase
- 2.4.1.89 *Now included with EC 2.4.1.69*
- 2.4.1.90 *N*-acetyllactosamine synthase
- 2.4.1.91 flavonol 3-*O*-glucosyltransferase
- 2.4.1.92 (*N*-acetylneuraminy)-galactosylglucosylceramide *N*-acetylgalactosaminyltransferase
- 2.4.1.93 inulin fructotransferase (depolymerizing, difructofuranose-1,2':2,3'-dianhydride-forming)
- 2.4.1.94 protein *N*-acetylglucosaminyltransferase
- 2.4.1.95 bilirubin-glucuronoside glucuronosyltransferase
- 2.4.1.96 *sn*-glycerol-3-phosphate 1-galactosyltransferase
- 2.4.1.97 1,3- β -D-glucan phosphorylase
- 2.4.1.98 *Now included with EC 2.4.1.90*
- 2.4.1.99 sucrose 1^F-fructosyltransferase
- 2.4.1.100 1,2- β -fructan 1^F-fructosyltransferase
- 2.4.1.101 α -1,3-mannosyl-glycoprotein 2- β -*N*-acetylglucosaminyltransferase

- 2.4.1.102 β -1,3-galactosyl-*O*-glycosyl-glycoprotein β -1,6-*N*-acetylglucosaminyltransferase
- 2.4.1.103 alizarin 2- β -glucosyltransferase
- 2.4.1.104 *o*-dihydroxycoumarin 7-*O*-glucosyltransferase
- 2.4.1.105 vitexin β -glucosyltransferase
- 2.4.1.106 isovitexin β -glucosyltransferase
- 2.4.1.107 *Now included with EC 2.4.1.17*
- 2.4.1.108 *Now included with EC 2.4.1.17*
- 2.4.1.109 dolichyl-phosphate-mannose—protein mannosyltransferase
- 2.4.1.110 tRNA-queuosine β -mannosyltransferase
- 2.4.1.111 coniferyl-alcohol glucosyltransferase
- 2.4.1.112 α -1,4-glucan-protein synthase (UDP-forming)
- 2.4.1.113 α -1,4-glucan-protein synthase (ADP-forming)
- 2.4.1.114 2-coumarate *O*- β -glucosyltransferase
- 2.4.1.115 anthocyanidin 3-*O*-glucosyltransferase
- 2.4.1.116 cyanidin-3-rhamnosylglucoside 5-*O*-glucosyltransferase
- 2.4.1.117 dolichyl-phosphate β -glucosyltransferase
- 2.4.1.118 cytokinin 7- β -glucosyltransferase
- 2.4.1.119 dolichyl-diphosphooligosaccharide—protein glycotransferase
- 2.4.1.120 sinapate 1-glucosyltransferase
- 2.4.1.121 indole-3-acetate β -glucosyltransferase
- 2.4.1.122 glycoprotein-*N*-acetylgalactosamine 3- β -galactosyltransferase
- 2.4.1.123 inositol 3- α -galactosyltransferase
- 2.4.1.124 *Now included with EC 2.4.1.87, N-acetyllactosaminide 3-alpha-galactosyltransferase.*
- 2.4.1.125 sucrose—1,6- α -glucan 3(6)- α -glucosyltransferase
- 2.4.1.126 hydroxycinnamate 4- β -glucosyltransferase
- 2.4.1.127 monoterpeneol β -glucosyltransferase
- 2.4.1.128 scopoletin glucosyltransferase
- 2.4.1.129 peptidoglycan glycosyltransferase
- 2.4.1.130 dolichyl-phosphate-mannose—glycolipid α -mannosyltransferase
- 2.4.1.131 glycolipid 2- α -mannosyltransferase
- 2.4.1.132 glycolipid 3- α -mannosyltransferase
- 2.4.1.133 xylosylprotein 4- β -galactosyltransferase
- 2.4.1.134 galactosylxylosylprotein 3- β -galactosyltransferase
- 2.4.1.135 galactosylgalactosylxylosylprotein 3- β -glucuronosyltransferase
- 2.4.1.136 gallate 1- β -glucosyltransferase

- 2.4.1.137 *sn*-glyc 3-phosphate 2- α -galactosyltransferase
2.4.1.138 mannotetraose 2- α -*N*-acetylglucosaminyltransferase
2.4.1.139 maltose synthase
2.4.1.140 altamansucrase
2.4.1.141 *N*-acetylglucosaminylidiphosphodolichol *N*-acetylglucosaminyltransferase
2.4.1.142 chitobiosylidiphosphodolichol β -mannosyltransferase
2.4.1.143 α -1,6-mannosyl-glycoprotein 2- β -*N*-acetylglucosaminyltransferase
2.4.1.144 β -1,4-mannosyl-glycoprotein 4- β -*N*-acetylglucosaminyltransferase
2.4.1.145 α -1,3-mannosyl-glycoprotein 4- β -*N*-acetylglucosaminyltransferase
2.4.1.146 β -1,3-galactosyl-*O*-glycosyl-glycoprotein β -1,3-*N*-acetylglucosaminyltransferase
2.4.1.147 acetylglactosaminyl-*O*-glycosyl-glycoprotein β -1,3-*N*-acetylglucosaminyltransferase
2.4.1.148 acetylglactosaminyl-*O*-glycosyl-glycoprotein β -1,6-*N*-acetylglucosaminyltransferase
2.4.1.149 *N*-acetylactosaminide β -1,3-*N*-acetylglucosaminyltransferase
2.4.1.150 *N*-acetylactosaminide β -1,6-*N*-acetylglucosaminyltransferase
2.4.1.151 *Now included with EC 2.4.1.67*
2.4.1.152 4-galactosyl-*N*-acetylglucosaminide 3- α -L-fucosyltransferase
2.4.1.153 dolichyl-phosphate α -*N*-acetylglucosaminyltransferase
2.4.1.154 globotriosylceramide β -1,6-*N*-acetylglactosaminyltransferase
2.4.1.155 α -1,6-mannosyl-glycoprotein 6- β -*N*-acetylglucosaminyltransferase
2.4.1.156 indolylacetyl-*myo*-inositol galactosyltransferase
2.4.1.157 1,2-diacylglycerol 3-glucosyltransferase
2.4.1.158 13-hydroxydocosanoate 13- β -glucosyltransferase
2.4.1.159 flavonol-3-*O*-glucoside L-rhamnosyltransferase
2.4.1.160 pyridoxine 5'-*O*- β -D-glucosyltransferase
2.4.1.161 oligosaccharide 4- α -D-glucosyltransferase
2.4.1.162 aldose β -D-fructosyltransferase
2.4.1.163 β -galactosyl-*N*-acetylglucosaminylgalactosylglucosylceramide β -1,3-acetylglucosaminyltransferase
2.4.1.164 galactosyl-*N*-acetylglucosaminylgalactosylglucosylceramide β -1,6-*N*-acetylglucosaminyltransferase
2.4.1.165 *N*-acetylneuraminylgalactosylglucosylceramide β -1,4-*N*-acetylglactosaminyltransferase

2.4.1.168 xyloglucan 4-glucosyltransferase
2.4.1.169 xyloglucan 6-xylosyltransferase
2.4.1.170 isoflavone 7-*O*-glucosyltransferase
2.4.1.171 methyl-*ONN*-azoxymethanol β -D-glucosyltransferase
2.4.1.172 salicyl-alcohol β -D-glucosyltransferase
2.4.1.173 sterol 3 β -glucosyltransferase
2.4.1.174 glucuronylgalactosylproteoglycan 4- β -*N*-acetylgalactosaminyltransferase
2.4.1.175 glucuronosyl-*N*-acetylgalactosaminyl-proteoglycan 4- β -*N*-acetylgalactosaminyltransferase
2.4.1.176 gibberellin β -D-glucosyltransferase
2.4.1.177 cinnamate β -D-glucosyltransferase
2.4.1.178 hydroxymandelonitrile glucosyltransferase
2.4.1.179 lactosylceramide β -1,3-galactosyltransferase
2.4.1.180 lipopolysaccharide *N*-acetylmannosaminouronosyltransferase
2.4.1.181 hydroxyanthraquinone glucosyltransferase
2.4.1.182 lipid-A-disaccharide synthase
2.4.1.183 α -1,3-glucan synthase
2.4.1.184 galactolipid galactosyltransferase
2.4.1.185 flavanone 7-*O*- β -glucosyltransferase
2.4.1.186 glycogenin glucosyltransferase
2.4.1.187 *N*-acetylglucosaminyldiphosphoundecaprenol *N*-acetyl- β -D-mannosaminyltransferase
2.4.1.188 *N*-acetylglucosaminyldiphosphoundecaprenol glucosyltransferase
2.4.1.189 luteolin 7-*O*-glucuronosyltransferase
2.4.1.190 luteolin-7-*O*-glucuronide 7-*O*-glucuronosyltransferase
2.4.1.191 luteolin-7-*O*-diglucuronide 4'-*O*-glucuronosyltransferase
2.4.1.192 nuatigenin 3 β -glucosyltransferase
2.4.1.193 sarsapogenin 3 β -glucosyltransferase
2.4.1.194 4-hydroxybenzoate 4-*O*- β -D-glucosyltransferase
2.4.1.195 thiohydroximate β -D-glucosyltransferase
2.4.1.196 nicotinate glucosyltransferase
2.4.1.197 high-mannose-oligosaccharide β -1,4-*N*-acetylglucosaminyltransferase
2.4.1.198 phosphatidylinositol *N*-acetylglucosaminyltransferase
2.4.1.199 β -mannosylphosphodecaprenol—mannooligosaccharide 6-mannosyltransferase
2.4.1.200 inulin fructotransferase (depolymerizing, difructofuranose-1,2':2',1-dianhydride-forming)
2.4.1.201 α -1,6-mannosyl-glycoprotein 4- β -*N*-acetylglucosaminyltransferase

2.4.1.202 2,4-dihydroxy-7-methoxy-2*H*-1,4-benz xazin-3(4*H*)-on
2-D-glucosyltransferase

2.4.1.203 *trans*-zeatin O- β -D-glucosyltransferase

2.4.1.204 zeatin O- β -D-xylosyltransferase

2.4.1.205 galactogen 6 β -galactosyltransferase

2.4.1.206 lactosylceramide 1,3-*N*-acetyl- β -D-
glucosaminyltransferase

2.4.1.207 xyloglucan:xyloglucosyl transferase

2.4.1.208 diglucosyl diacylglycerol synthase

2.4.1.209 *cis*-*p*-coumarate glucosyltransferase

2.4.1.210 limonoid glucosyltransferase

2.4.1.211 1,3- β -galactosyl-*N*-acetylhexosamine phosphorylase

2.4.1.212 hyaluronan synthase

2.4.1.213 glucosylglycerol-phosphate synthase

2.4.1.214 glycoprotein 3- α -L-fucosyltransferase

2.4.1.215 *cis*-zeatin O- β -D-glucosyltransferase

2.4.1.216 trehalose 6-phosphate phosphorylase

2.4.1.217 mannosyl-3-phosphoglycerate synthase

2.4.1.218 hydroquinone glucosyltransferase

2.4.1.219 vomilenine glucosyltransferase

2.4.1.220 indoxyl-UDPG glucosyltransferase

2.4.1.221 peptide-O-fucosyltransferase

2.4.1.222 O-fucosylpeptide 3- β -*N*-acetylglucosaminyltransferase

2.4.1.223 glucuronyl-galactosyl-proteoglycan 4- α -*N*-
acetylglucosaminyltransferase

2.4.1.224 glucuronosyl-*N*-acetylglucosaminyl-proteoglycan 4- α -*N*-
acetylglucosaminyltransferase

2.4.1.225 *N*-acetylglucosaminyl-proteoglycan 4- β -
glucuronosyltransferase

2.4.1.226 *N*-acetylgalactosaminyl-proteoglycan 3- β -
glucuronosyltransferase

2.4.1.227 undecaprenyldiphospho-muramoylpentapeptide β -*N*-
acetylglucosaminyltransferase

2.4.1.228 lactosylceramide 4- α -galactosyltransferase

2.4.1.229 [Skp1-protein]-hydroxyproline *N*-
acetylglucosaminyltransferase

2.4.1.230 kojibiose phosphorylase

2.4.1.231 α , α -trehalose phosphorylase (configuration-retaining)

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APPENDIX B:

Lectins available from Sigma-Aldrich: 2002-2003 catalog

1224

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Lect

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APPLIED BIOTECHNICAL LIST OF PRODUCTS

Lect

US \$	<p>L 2886 [E3E]</p> <p>Lectin from <i>Lycopersicon esculentum</i> (tomato) Lyophilized powder Lycopodium lectin is a glycoprotein with an affinity for D-glucose oligomers. The lectin is a glycoprotein containing approx. equal amounts of protein and carbohydrate and is reported to inhibit the mitogenic activity of phytohemagglutinin from <i>Phaseolus vulgaris</i>. Contains NaCl Purified by affinity chromatography. Agglutination activity is expressed in µg per ml and is determined from serial dilutions in phosphate buffered saline, pH 7.3, of a 1 mg per ml solution. This activity is the lowest concentration to agglutinate a 2% suspension of human erythrocytes after 1 hr incubation at 25 °C. Potency: <2 µg per ml. Lectin minimum 40%</p>	1 mg 42.40 5 mg 166.90	<p>L 2033 [E3E]</p> <p>Lectin, Biotin labeled (orange) from <i>Macharia pomifera</i> (Osage orange) Lyophilized powder Contains sodium citrate Package size based on protein content. Agglutination activity is expressed in µg per ml and is determined from serial dilutions in phosphate buffered saline, pH 6.8, of a 1 mg per ml solution. This activity is the lowest concentration to agglutinate a 2% suspension of human erythrocytes after 1 hr incubation at 25 °C. Protein approx. 85% by 1% assay Lectin of labeling ... approx. 2 mg biotin per mg protein</p>	<p>L 16199 [E3E]</p> <p>Erythroagglutinin PHA-E, TRIC from <i>Phaseolus vulgaris</i> (red kidney bean) Lyophilized powder Contains phosphate buffer salts and NaCl Package size based on protein content. Protein approx. 10% by Lowry Lectin of labeling ... approx. 1 mg TRIC per mg protein</p>	<p>L 2646 [E3E]</p> <p>Phytoagglutinin PHA-M from <i>Phaseolus vulgaris</i> (red kidney bean) Lyophilized powder Lectin activity is expressed in µg per ml and is determined from serial dilutions of a 1 mg per ml solution. This activity is the lowest concentration to agglutinate a suspension of either human erythrocytes (2% in phosphate buffered saline, pH 6.8) or human leukocytes (10% per ml in saline) after 1 hr incubation at 25 °C. Mitogenic activity is determined by ³H-thymidine incorporation in lymphocyte cultures. Potency: <40 µg per ml (with erythrocytes) or <10 µg per ml (with lymphocytes) Lectin of labeling ... approx. 5 mg 21.70 25 mg 36.60 100 mg 118.10</p>
	<p>L 4401 [E3E]</p> <p>Lectin, Peroxidase labeled (orange) from <i>Macharia pomifera</i> (Osage orange) Lyophilized powder Contains sodium citrate Prepared from peroxidase labeled using a modification of the method of O'Sullivan, M.J., et al., FEBS Lett., 95, 311 (1978), which favors low molecular weight conjugates. Purified by affinity chromatography after conjugation. Package size based on protein content. Unit definition: One unit will form 1 mg peroxidase from D-tryptophan in 20 sec at pH 6.0 at 20 °C. Agglutination activity is expressed in µg per ml and is determined from serial dilutions in phosphate buffered saline, pH 6.8, of a 1 mg per ml solution. This activity is the lowest concentration to agglutinate a 2% suspension of human erythrocytes after 1 hr incubation at 25 °C. Protein approx. 90% by modified Warburg-Chicklin</p>	1 mg 130.50	<p>L 3007 [E3E]</p> <p>Lectin-Agarose from <i>Phaseolus vulgaris</i> (red kidney bean) Sulfate suspension Supernatant in 1.0 M NaCl Lectin of labeling ... approx. 2.4 mg per ml</p>	<p>L 2768 [E3E]</p> <p>Leucoagglutinin PHA-L Purified Lyophilized powder Purified by a modification of the method of Leavitt, R.D., et al., Biol. Chem., 252, 2951 (1977). Tested for leucoagglutination. Does not agglutinate erythrocytes at 250 µg per ml. Mitogenic activity is determined by ³H-thymidine incorporation in lymphocyte cultures. Potency: <10 µg per ml (with erythrocytes) or <10 µg per ml (with lymphocytes) Lectin of labeling ... approx. 1 ml 77.20 2 ml 128.35</p>	<p>L 8802 [E3E]</p> <p>Phytohemagglutinin PHA-M from <i>Phaseolus vulgaris</i> (red kidney bean) Lyophilized powder, cell culture tested Mitogenic activity: <10 µg per ml Lectin of labeling ... approx. 5 mg 26.60 25 mg 40.90 100 mg 128.50</p>
	<p>L 0651 [E3E]</p> <p>Lectin, Biotin labeled (tomato) from <i>Lycopersicon esculentum</i> (tomato) Lyophilized powder Contains NaCl Lectin of labeling ... approx. 1 mg 56.05 2 mg 100.90</p>	1 mg 56.05 2 mg 100.90	<p>L 0917 [E3E]</p> <p>Phytohemagglutinin from <i>Phaseolus vulgaris</i> (red kidney bean) Lyophilized powder Lectin from <i>Phaseolus vulgaris</i> (red kidney bean) Phytolhemagglutinin PHA-P Lyophilized powder Mitogenic activity: <10 µg per ml Lectin of labeling ... approx. 1 mg 22.95 5 mg 83.45 10 mg 149.70</p>	<p>L 4144 [E3E]</p> <p>Leucoagglutinin PHA-L from <i>Phaseolus vulgaris</i> (red kidney bean) Lyophilized powder, cell culture tested, leucocyte agglutination activity tested Purified Mitogenic concentration: <5 µg per ml Lectin of labeling ... approx. 5 mg 137.30</p>	<p>L 1931 [E3E]</p> <p>Phytohemagglutinin PHA-P from <i>Phaseolus vulgaris</i> (red kidney bean) Lyophilized powder, cell culture tested Agglutination activity: <16 µg per ml (with erythrocytes) Potency: <16 µg per ml (with erythrocytes) or <10 µg per ml (with lymphocytes) Lectin of labeling ... approx. 5 mg 25.55</p>
	<p>L 0401 [E3E]</p> <p>Lectin, Biotin labeled (tomato) from <i>Lycopersicon esculentum</i> (tomato) Lyophilized powder Contains NaCl Lectin of labeling ... approx. 1 mg 85.80</p>	1 mg 85.80	<p>L 0654 [E3E]</p> <p>Erythroagglutinin PHA-E from <i>Phaseolus vulgaris</i> (red kidney bean) Lyophilized powder Contains NaCl Purified by a modification of the method of Leavitt, R.D., et al., Biol. Chem., 252, 2951 (1977). Tested for leucoagglutination. Agglutination activity is expressed in µg per ml and is determined from serial dilutions of a 1 mg per ml solution. This activity is the lowest concentration to agglutinate a suspension of either human erythrocytes (2% in phosphate buffered saline, pH 6.8) or human leukocytes (10% per ml in saline) after 1 hr incubation at 25 °C. Mitogenic activity is determined by ³H-thymidine incorporation in lymphocyte cultures. Potency: <10 µg per ml (with erythrocytes) or <10 µg per ml (with lymphocytes) Lectin of labeling ... approx. 5 mg 25.55</p>	<p>L 1938 [E3E]</p> <p>Phytohemagglutinin PHA-P from <i>Phaseolus vulgaris</i> (red kidney bean) Lyophilized powder Mitogenic activity: <10 µg per ml Lectin of labeling ... approx. 1 mg 25.40</p>	<p>L 1938 [E3E]</p> <p>Phytohemagglutinin PHA-P from <i>Phaseolus vulgaris</i> (red kidney bean) Lyophilized powder Mitogenic activity: <10 µg per ml Lectin of labeling ... approx. 1 mg 25.40</p>

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1231

(Continuation of)		Lectin	
L 4389 [533]	Lectin, TRITC labeled from <i>Phaseolus coccineus</i> (Scarlet Runner Bean) Lyophilized powder Contains phosphate buffer salts and NUC1 Package size based on protein content. Agglutination activity is expressed in µg/ml and is determined from serial dilutions in phosphate buffered saline, pH 6.9, of 1 mg/ml solution. This activity is the lowest concentration to agglutinate a 2% suspension of human erythrocytes after 1 hr incubation at 25°C. Histoergic activity is determined by ³ H-thymidine incorporation in lymphocyte cultures. Protein approx. 10% by Lowry Content of labeling: 1-2 µCi of TRITC per mg protein	5 mg 149.80	
Lectin	from <i>Phytolacca americana</i> (pokeweed) Fetowed nitrogen, PAM PAM has hemagglutinating, leukoagglutinating and mitogenic properties. PAM is not blood group specific, but has ability for N-acetyl-β-D-glucosamine oligomers. Rec. Yabumori, K. et al., <i>J. Biol. Chem.</i> 253, 334 (1978) p. 43-5; 26-36	5 mg 43.05 10 mg 71.55 40 mg 231.55	
L 9379 [532]	Lectin from <i>Phytolacca americana</i> (pokeweed) Lyophilized powder [63231-57-2] Partially purified. Nitrogenic at approx. 2.5 µg per ml. Lyophilized TCA precipitate Agglutination activity is expressed in µg per ml and is determined from serial dilutions in phosphate buffered saline, pH 6.8, of a 1 mg per ml solution. This activity is the lowest concentration to agglutinate a 2% suspension of human blood group A erythrocytes after 1 hr incubation at 25°C. Mitogenic activity was determined by ³ H-thymidine incorporation in lymphocyte cultures. asceptically filled Potency: <20 µg per ml	5 mg 62.75 10 mg 62.30	
L 9377 [532]	Lectin from <i>Phytolacca americana</i> (pokeweed) Lyophilized powder, cell culture tested [63231-57-2] Partially purified TCA precipitate. Essentially salt-free. Aseptically filled Agglutination activity: <20 µg per ml Mitogenic activity: approx. 2.5 µg per ml salt Solubility pH 5 essentially free pH 8 1 mg/ml		
L 2881 [535]	Lectin-Agarose from <i>Phytolacca americana</i> (pokeweed) Suspension in 1.0 M NaCl Assay of blood group Matrix action cross-linked 4% beefed soybean Matrix action O antigenic blood Matrix action O antigenic blood Matrix action O antigenic blood	1 ml 43.90	

<p>Lectin from <i>Pisum sativum</i> (pea) Peanut agglutinin (PNA) is not blood group specific, but has an affinity for terminal α-mannosyl residues. PNA lectin is a mitogen similar to concanavalin A. Conjugates are prepared from affinity purified lectin. Agglutination activity is expressed in μg per ml and is determined from serial dilutions in phosphate buffered saline, pH 6.8, of a 1 mg per ml solution. This activity is the lowest concentration to agglutinate a 2% suspension of human erythrocytes after 1 hr incubation at 25°C. Protein approx. 15% by modified Warburg-Christian method. Ref.: Towb, J. S., J. Biol. Chem. 259, 6034 (1984) B-4203, 5, 36</p>	<p>Lectin from <i>Rhizopus communis</i> (castor bean) Reticular lectin (RLC) occurs in two forms designated RLC_{AP} and RLC_{AB} according to their mol. wt. of approx. 60,000 and 120,000 respectively. Neither RLC_{AP} nor RLC_{AB} is blood group specific. RLC_{AP} also referred to as RLC₂ or RLC_{II} is extremely toxic, inhibits protein synthesis and has an affinity for N-acetylglucosamine. RLC_{AB} also referred to as RLC₁ or RLC_I is a non-toxic lectin and has an affinity for terminal D-galactosyl residues. Conjugates are prepared from affinity purified lectin. RCA_{AP} or agglutinin RCA_{AB}. WARNING: Extremely hazardous! Be aware of the risks and families with safety procedures before you use this product. Ref.: 1. Nicholson, L. J., et al., Biochemistry 13, 156 (1974) 2. U. T. S., U. S. G. I., Fed. J. Govt. Serv. 105, 453 (1960) 3. Ibrahim, G. I., Bashir, J., British Med. J. 286, 543 (1978) 4. NKS (C.H.), M.O. C., J. Med. Biol. 123, 709 (1978)</p>	<p>Lectin from <i>Rhizopus communis</i> (castor bean) Reticular lectin (RLC) occurs in two forms designated RLC_{AP} and RLC_{AB} according to their mol. wt. of approx. 60,000 and 120,000 respectively. Neither RLC_{AP} nor RLC_{AB} is blood group specific. 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L. 2285
[L285] ◆
Agglutinin RCA₁₂₀
from *Rhus communis* (castor bean)
Buffered aqueous solution
Solution in 0.005 M sodium phosphate and 0.2 M sodium chloride, pH 7.2, containing 0.1% sodium azide.
Purified by affinity chromatography using the method of U.S. and Li, S.Z., *in J. Biochem.*, 105, 453 (1980).
Agglutination activity is expressed in units and determined from serial dilutions in phosphate buffered saline, pH 7.2, of a 1 mg protein per ml solution. This activity is the lowest concentration to agglutinate a 2% suspension of human erythrocytes after 1 hour incubation at 25°C.
Potency: <1 µg per ml.
R 4243-267708 p. 53-56-45

L. 22758
[258] ◆
Lactin, Peroxidase labeled,
Agglutinin RCA₁₂₀
from *Rhus communis* (castor bean)
Lyophilized powder
Conlat's soil mix dries
Prepared by peroxidase type VI using a two step glutaraldehyde method of Ameress, S. and Tirmody, T., *Inmunochimistry*, 8, 1175 (1971).
Repurified after conjugation by affinity chromatography.
Use definition: One unit will form 1 mg purpurogallin in 20 sec. at room temp. at pH 6.0 at 20°C.
Protein approx. 75%, by modified Weibull-Christian R 4243-267708 p. 53-56-45

Lectin-Agarose
2390
[239] ◆
Lectin from Ricinus communis (castor bean)
Saline suspension
Ricinus communis agglutinin (RCA) occurs in two forms designated RCA₅₀ and RCA₁₂₀ according to their mol. wt. of approx. 60,000 and 120,000 respectively. Neither RCA₅₀ nor RCA₁₂₀ is blood group specific. RCA also referred to as RCA, Ricin D⁺ or RCA II is extremely toxic, inhibits protein synthesis and has an affinity for Haeβ-glycoalbumin. RCA₅₀ absorbed to as RCA₂ or RCA I + II is an agglutinin and has an affinity for terminal β-D-glucosyl residues.
Conjugates are prepared from affinity purified toxin, RCA₅₀ or agglutinin RCA₁₂₀.
WARNING: Extremely hazardous! Be aware of the risks and familiar with safety procedures before you use this product.
Suspension in 1.0 M NaCl containing 0.00% (W/V) coal and 0.02% sodium azide
Pack or loting 2-4 mg protein per mL
Media action Does not bind α₁-branched oligo saccharides
Alkaline action Phosphatidylethanolamine
RBC action
RBC 1. Redden G.J., et al., *Biochemistry* 13, 195 (1974)
2. Wu, L.S., Li, S.Z., *in J. Biochem.* 105, 453 (1980)
3. Redden G.J., *in J. Biol. Chem.* 255, 453 (1980)
4. Wu, C.H., Liu, C. L., *J. Biol. Chem.* 255, 707 (1980)

Lectin
from *Tetragonia bicolor purpureus* (Cortus tetragonoloba), winged or asparagus pea)
Tetragonia purpureus agglutinin (Tos agglutinin) has anti-H blood group specificity. The lectin has an affinity for α-L-fucose residues and an unusually high affinity for α-L-fucose residues on type B chain blood group oligosaccharides.

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Lect 10

(Continuation of)		Lectin	
L 9154 [32E]	Lyophilized powder purified by affinity chromatography. Agglutination activity is expressed in µg per ml and is determined from serial dilutions in phosphate buffered saline, pH 6.8, at a 1 mg per ml solution. This activity is the lowest concentration to agglutinate a 2% suspension of human blood group O erythrocytes after 1 hr incubation at 25°C. Potency: <37 µg per ml.	5 mg	70.00
L 3134 [32E]	Lectin, Biotin labelled from <i>Tetragonolobus purpureus</i> (Lonus tetragonolobus, winged or asparagus pea). Lyophilized powder. Particle size based on protein content. Contains sodium citrate. Protein approx. 85% by [1] 250. Loss of activity approx. 6 and 10 min per ml per hour at 25°C.	1 mg	98.95
L 5644 [32E]	Lectin, FITC labelled from <i>Tetragonolobus purpureus</i> (Lonus tetragonolobus, winged or asparagus pea). Lyophilized powder. Enzyme of labelling: 2-6 mol FITC per mol protein.	1 mg	54.10
L 5719 [32E]	Lectin, Peroxidase labelled from <i>Tetragonolobus purpureus</i> (Lonus tetragonolobus, winged or asparagus pea). Lyophilized powder. Containing sodium citrate. Prepared from peroxidase type VI using a modification of the method of O'Sullivan, M.J., et al., 1955. Lot: 98, 311 (1978) which factors are molecular weight conjugates, reagent quality conjugation by affinity chromatography. Package size based on protein content. Unit definition: One unit will form 1 µg peroxidase in 20 sec. from pyrogallol at pH 6.0 at 20°C. Protein approx. 90% by modified Warburg-Chibrian	250 µg	47.55
L 5719 [32E]	Lectin, Peroxidase labelled from <i>Tetragonolobus purpureus</i> (Lonus tetragonolobus, winged or asparagus pea). Lyophilized powder. Containing sodium citrate. Prepared from peroxidase type VI using a modification of the method of O'Sullivan, M.J., et al., 1955. Lot: 98, 311 (1978) which factors are molecular weight conjugates, reagent quality conjugation by affinity chromatography. Package size based on protein content. Unit definition: One unit will form 1 µg peroxidase in 20 sec. from pyrogallol at pH 6.0 at 20°C. Protein approx. 90% by modified Warburg-Chibrian	1 mg	128.80
L 9540 [32E]	Lectin from <i>Trifolium vulgare</i> . Whole germ agglutinin (WGA) is not blood group specific, but has an affinity for N-acetyl-β-D-glucosaminyl residues and N-acetyl-β-D-glucosamine oligomers. WGA contains no protein-bound carbohydrate. Trifolium vulgare wheat germ conjugates are prepared from affinity purified lectin.	10 mg	67.35
L 9540 [32E]	Lectin from <i>Trifolium vulgare</i> . Whole germ agglutinin (WGA) is not blood group specific, but has an affinity for N-acetyl-β-D-glucosaminyl residues and N-acetyl-β-D-glucosamine oligomers. WGA contains no protein-bound carbohydrate. Trifolium vulgare wheat germ conjugates are prepared from affinity purified lectin.	25 mg	122.30
L 9540 [32E]	Lectin from <i>Trifolium vulgare</i> . Whole germ agglutinin (WGA) is not blood group specific, but has an affinity for N-acetyl-β-D-glucosaminyl residues and N-acetyl-β-D-glucosamine oligomers. WGA contains no protein-bound carbohydrate. Trifolium vulgare wheat germ conjugates are prepared from affinity purified lectin.	100 mg	373.40
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L 1882 (23)	Lectin-Agarose from <i>Triticum vulgare</i> Saline suspension Wheat germ agglutinin, immobilized on agarose CL-4B Sugar specificity: D-N-acetylglucosamine, N-acetylgalactosaminic acid Suspension in 1.0 M NaCl containing 0.02% thinnex	1 ml 107.25 5 ml 320.25 10 ml 533.68
L 1944 (23)	Lectin-Agarose from <i>Triticum vulgare</i> 200-300 µm, saline suspension Used for the purification of glycoproteins containing D-N-acetylglucosamine or N-acetylgalactosaminic acid Sugar specificity: D-N-acetylglucosamine, N-acetylgalactosaminic acid Suspension in 0.9% NaCl containing 0.01% thinnex	1 ml 63.50 5 ml 238.70 10 ml 473.95
L 4257 (23)	Lectin-Sephacrose 6MB from <i>Triticum vulgare</i> 200-300 µm, saline suspension Sugar specificity: D-N-acetylglucosamine, N-acetylgalactosaminic acid Suspension in 0.9% NaCl and 0.0% thinnexol	1 ml 137.85 5 ml 459.45 10 ml 765.75
L 5142 (23)	Lectin, Biotin labeled from <i>Triticum vulgare</i> Lyophilized powder Contains sodium citrate Package size based on protein content Protein approx. 85%	1 mg 27.85 5 mg 86.65
L 5884 (23)	Lectin, Evans Blue labeled from <i>Triticum vulgare</i> Lyophilized powder Contains ribitol buffer salts Package size based on protein content Protein approx. 85%	1 mg 49.15
L 4895 (23)	Lectin, FITC labeled from <i>Triticum vulgare</i> Lyophilized powder Contains phosphate buffer salts Package size based on protein content Protein approx. 50% by Lowry	2 mg 39.85 5 mg 79.38 10 mg 144.78
L894 (23)	10 nm colloidal gold, approx. 5 A ₂₁₀ units/ml, aqueous glycerol suspension Suspension in approx. 50% glyceral containing 0.15 M NaCl, 0.01 M sodium phosphate, pH 7.0, 0.02% PEG 20, and 0.02% sodium azide	0.2 ml 28.97 1 ml 94.55

<p>L2932 293</p> <p>Lectin, Peroxidase labeled from <i>Triticum vulgare</i> Lyophilized powder Contents: 0.8 ml buffer Prepared from peroxidase type VI using a modification of the method of O'Sullivan, M.J., et al., FEBS Lett. 95, 311 (1978) which favors low molecular weight conjugates. Recupitated after conjugation by affinity chromatography. Package size based on protein content Unit definition: One unit will form 1.0 mg of purpogallin from purpogallin in 20 sec at pH 6.0 at 20°C. Protein approx. 90% by modified Warburg-Christian § 72-2425</p>	<p>1 mg 89.15 2 mg 147.25 5 mg 313.50</p>
<p>L2937 293</p> <p>Lectin, Peroxidase labeled from <i>Triticum vulgare</i> Package size based on protein content Same as L2932, but packaged in microcentrifuge vials for ease of reconstruction and economy of microtiter volumes</p>	<p>500 µg 85.70 1 mg 141.35</p>
<p>L2939 293</p> <p>Lectin, Peroxidase, inactivated, from <i>Triticum vulgare</i> Lyophilized powder Potentially useful as an alternate probe in dual neuronal transport studies where labeled with an appropriate marker. Contains up to 10% lactate Prepared from inactivated peroxidase; P 6278, using a modification of the method of O'Sullivan, M.J., et al., FEBS Lett. 95, 311 (1978). Package size based on protein content Unit definition: One unit will form 1 mg purpogallin in 20 sec at purpogallol at pH 6.0 at 20°C. Protein approx. 50% by modified Warburg-Christian Peroxidase Assay § 72-2425</p>	<p>250 µg 52.00 500 µg 85.15 1 mg 133.25</p>
<p>L2956 293</p> <p>Lectin, TRITC labeled from <i>Triticum vulgare</i> Lyophilized powder Contents: phosphate buffer, salts and NaCl Package size based on protein content Protein approx. 10% by Lowry Front of labeling approx. 1 mol TRITC per mol protein</p>	<p>2 mg 97.10</p>
<p>L2982 293</p> <p>Lectin-Agarose from <i>Ulex europaeus</i> (gum), Ulex A-I Saline suspension Ulex europaeus agglutinin (UEA) has anti-B blood group specificity. There are two types of lectin UEA-I has an affinity for fucose and UEA-II has an affinity for N,N'-diacetylchitosamine. The mol. wt. of UEA I was initially found to be 170,000. Later reports indicate that UEA I may form aggregates at neutral and basic pH and that the correct mol. wt. is 68,000. Suspension in 1.0 M NaCl (with 0.01% thimerosal) of labeling 2.4 mg per ml Bottle capacity: 85.4 mg/ml approx. 3 mg/ml Ref.: L. Allen, H.J. Allen, J. Nat. Cancer Inst. 58, 253 (1977) 2. Hregal, V. Kozaruk, J. Biochem. Biophys. Acta 218, 279 (1974) 3. Malmstrom, L. Osawa, I., Biochim. Biophys. Acta 194, 180 (1976)</p>	<p>1 ml 60.55 2 ml 100.55</p>

L 8146 [C91]	Lectin, Peroxidase labeled (from <i>Ulex europaeus</i> (gorse, furze))	500 µg 1 mg	70.50 118.40
	Lyophilized powder Contains sodium citrate buffer salts Prepared from peroxidase, Type VI, using the two-step glutaraldehyde method of Awanaka, S. and Terayak, T., <i>Immunochemistry</i> , 8, 1175 (1971). Repurified by affinity chromatography after conjugation		
	Package size based on protein content. Unit definition: One unit will form 1.0 mg purpurogallin in 20 sec from purpurogallin at pH 6.0 at 20°C. Protein approx. 80% by Lowry		
L 9005 [C91]	Lectin, FITC labeled (from <i>Ulex europaeus</i> (gorse, furze))	1 mg 2 mg	54.40 90.60
	Lyophilized powder Contains potassium phosphate buffer salts and NaCl Package size based on protein content. Protein approx. 100% by Lowry Form of labeling 75 mol FITC per mol protein		
L 8262 [C91]	Lectin, Biotin labeled (from <i>Ulex europaeus</i> (gorse, furze))	500 µg 2 mg	38.00 87.20
	Lyophilized powder Contains sodium citrate Package size based on protein content. Protein approx. 85% by E_{280} Form of labeling approx. 3 mol biotin per mol protein		
L 5505 [C91]	Lyophilized powder Purified by affinity chromatography using the method of Fenech, M. T. A., 10 mg 95.00 et al., <i>Arch. Biochem. Biophys.</i> , 185, 105 (1978).	2 mg 5 mg	53.55 95.00
	Agglutination activity is expressed in µg per ml and is determined from serial dilutions in phosphate buffered saline, pH 6.8, of a 1 mg per ml solution. This activity is the lowest concentration to agglutinate a 2% suspension of human blood group O erythrocytes after 1 hr incubation at 25°C. Potency: <8 µg per ml		
	Form of labeling essentially free		
L 4889 [C91]	Lectin, TRITC labeled (from <i>Ulex europaeus</i> (gorse, furze))	500 µg	96.05
	Lyophilized powder Contains potassium phosphate buffer salts and sodium chloride Package size based on protein content Protein approx. 100% by Lowry Form of labeling 4.8 mol TRITC per mol lectin		
	from <i>Ulex europaeus</i> (gorse, furze) UEA-I Ulex europaeus agglutinin (UEA) has anti-H blood group specificity. There are two types of lectin: UEA I has an affinity for L-fucose and UEA II has an affinity for N-acetylglucosamine. The mol. wt. of UEA I was initially found to be 170,000. Later reports indicate that UEA I may form aggregates at neutral and basic pH and that the correct mol. wt. is 68,000. Ref.: 1. Allen, H., Johnson, L.A., <i>Cytochrome</i> , 25, 253 (1977). 2. Hoppel, V., Kozurek, J., <i>Biochim. Biophys. Acta</i> 336, 329 (1974). 3. Matsunaga, I., Osewa, T., <i>Biochim. Biophys. Acta</i> 194, 180 (1969).		

Let

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Docket 096/004

APPENDIX C:

Complement available from Sigma-Aldrich: 2002-2003 catalog

f Products

www.sigma-aldrich.com

Complement

100 µg 114.00

in 10 mM
00,000 C6H50 units/
ve hemolytic assay,
um 1 mL 89.10

orption
hemo-
122, 2103 (1979)
100 µg 91.50

in 10 mM
100,000 C7H50 units/
ive hemolytic assay,
um 1 mL 93.80

sorption
e hemo-
122, 2103 (1979)
100 µg 109.15

1
pH 7.2,
units/mg protein
suitable for radio-
ve hemolytic assay using
um 1 mL 96.65

isorption
ve hemo-
122, 2103 (1979)
100 µg 100.10

m
pH
50 units/mg (using C9
ive hemolytic assay using
um 1 mL 96.65

dsorption
ive hemo-
122, 2103 (1979)
100 µg 100.10

100 µg 136.75
1 mg 257.95

C1s, in
C1 complex which is the
sical complement path-
composed of 18
x B, Six C(MW 460kd). All
in an 81-aa collagen-like
(aa-Yaa) repeating
minus. These three types

of chains interact in sets of three (A,B,C) to form a triple helix with the C-terminus forming the globular heads which may be structurally and functionally distinct domains. One C1q molecule is comprised of six triple helices.
Lyophilized from 0.05 M Tris buffer, pH 7.3, containing 0.5 M NaCl
Pkg based on weight of protein
Single band by immunoelectrophoresis at 20 µg protein per gel against anti-C1q serum; single or no band against anti-whole serum. No visible reaction against anti-IgG, anti-IgM and anti-IgA sera at 20 µg protein per gel.
Ref.: 1. Tas, S.W., et al., C1q and C4b bind simultaneously to CR1 and additively support erythrocyte adhesion. *J. Immunol.* 163, 5056-5063 (1999)
2. Navratil, J.S., et al., Systemic lupus erythematosus and complement deficiency: clues to a novel role for the classical complement pathway in the maintenance of immune tolerance. *Immunopharmacology* 42, 47-52 (1999)
3. Kishore, U. and Reid, K. B., Modular organization of proteins containing C1q-like globular domain *Immunopharmacology* 42, 15-21 (1999)
4. Ruiz, S., et al., Digestion of C1q collagen-like domain with MMPs-1, -2, -3, and -9 further defines the sequence involved in the stimulation of neutrophil superoxide production. *J. Leukoc. Biol.* 66, 416-422 (1999)

Complement factor D 10 µg 123.30
C 5688
[C-5688]
DRY ICE
from human plasma
[37213-56-2]
>90% (SDS-PAGE), 100 µg/mL in
PBS, pH 7.2
Ref.: Niemann, M.A., et al., *J. Immunol.* 132, 809 (1984)

Complement factor H 100 µg 115.75
C 5813
[C-5813]
DRY ICE
from human plasma
[80295-65-4]
>90% (SDS-PAGE), 1 mg/mL in
PBS, pH 7.2
C3b-binding protein which regulates the formation and function of complement C3 and C5 convertases.
Ref.: 1. Fearon, D.T. and Austen, K.F., *Proc. Natl. Acad. Sci. USA* 74, 1583 (1977)
2. Pangburn, M.K. and Muller-Eberhard, H.J., *Springer Semin. Immunopath.* 7, 63 (1984)

Complement factor I 100 µg 202.50
C 5938
[C-5938]
DRY ICE
from human plasma
[80295-66-5] EC 3.4.21.45
>90% (SDS-PAGE), 1 mg/mL in
PBS, pH 7.2
Protease which cleaves and inactivates C3b and C4b
Ref.: Pangburn, M.K., et al., *J. Exp. Med.* 146, 257 (1977)

Complement sera

S 1639
[C-1639]
DRY ICE
Complement sera from guinea pig
Lyophilized powder
Lyophilized powder from indicated amount of serum
Hemolytic titer (CH50 units per ml) is determined by method of Kabat and Mayer. Actual titer given on label.
Ref.: Kabat, E.A. and Mayer, M.M., *Experimental Immunology* 2nd ed., Springfield, Illinois (1961),

S 1764
[C-1764]
DRY ICE

Complement sera human

1 mL 35.55
5 x 1 mL 68.15
Lyophilized powder
Lyophilized powder from indicated amount of serum
Hemolytic titer (CH50 units per ml) is determined by method of Kabat and Mayer. Actual titer given on label.
Ref.: Kabat, E.A. and Mayer, M.M., *Experimental Immunology* 2nd ed., Springfield, Illinois (1961).

S 3269
[C-3269]
DRY ICE

Complement sera from mouse

1 mL 69.30
Lyophilized powder
Lyophilized powder from indicated amount of serum
Hemolytic titer (CH50 units per ml) is determined by the method of Kabat and Mayer. Actual titer given on the label.
Ref.: Kabat, E.A. and Mayer, M.M., *Experimental Immunology* 2nd ed., Springfield, Illinois (1961).

S 7764
[C-7764]
DRY ICE

Complement sera from rabbit

1 mL 46.15
5 mL 60.25
Lyophilized powder
Lyophilized powder from indicated amount of serum

S 3394
[C-3394]
DRY ICE

Complement sera from rat

1 mL 57.80
5 x 1 mL 149.50
Lyophilized powder
Lyophilized powder from indicated amount of serum
Hemolytic titer (CH50 units per ml) is determined by the method of Kabat and Mayer. Actual titer given on the label.
Ref.: Kabat, E.A. and Mayer, M.M., *Experimental Immunology* 2nd ed., Springfield, Illinois (1961).

C 9473
[C-9473]
DRY ICE

Complement sera Complement Serum Standard human

1 mL 117.10
Aqueous solution
Substrate serum used as standard for quantization of components C1q, C2, C3, C4, C5, C6, C7, C8, C9 and factor B.
Solution containing 10 mM EDTA
Classical pathway activity (CH50 units per ml), alternative pathway activity (AH50 units per ml), antigen levels by radioimmunoassay and functional activities by hemolytic assay are provided in data sheet.

Complement assay reagents

G 6514
[C-6514]
Gelatin veronal buffer 50 mL 12.40
GVB²⁺ 5 x 50 mL 54.90
0.15 mM CaCl₂, 141 mM NaCl,
0.5 mM MgCl₂, 0.1% gelatin, 1.8 mM sodium barbitol and 3.1 mM barbituric acid, pH 7.3-7.4.
Aseptically filtered

G 9660
[C-9660]
Gelatin veronal buffer-EDTA 50 mL 11.40
GVB-EDTA 5 x 50 mL 50.50
Contains 141 mM NaCl, 0.1% gelatin, 1.8 mM sodium barbitol, 3.1 mM barbituric acid and 10 mM EDTA, pH 7.3-7.4.
Aseptically filtered

E 9383
[C-9383]
WET ICE
Antibody Sensitized Sheep Erythrocytes 2 mL 225.00
EA75 5 x 2 mL 749.90
1 x 10⁹ cells/mL, buffered aqueous cell suspension, suitable for assay of complement component activity (H50 units) and whole complement activity (CH50 units)
Suspension in gelatin veronal buffer containing 0.1 M sucrose

Complexone 1st Sec: Nitrilotriacetic acid Page 1516

Complexone V&B See: Diethylenetriaminepentaacetic acid Page 677

geron

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